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Infection Experiments with Spindle Tuber and Unmottled Curly Dwarf of the Potato

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SUMMARY

I. It was found by surveys of potato fields in western Nebraska that insects are not so abundant in dry-land as in irrigated fields, that grasshoppers are most widely distributed, and that flea-beetles are the most serious pests in certain local areas. In addition, it was found that aphids sometimes occur in irrigated fields in large enough numbers to be of importance in the spread of virus diseases.

2. Experimental tests proved that both spindle tuber and unmottled curly dwarf could be transmitted by grasshoppers (*Melanoplus* spp.); the flea-beetles (*Epitrix cucumeris* and *Systena taeniata*); the tarnished plant bug (*Lygus pratensis*); and the larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*). The leaf beetle (*Disonycha triangularis*) transmitted spindle tuber but failed to transmit unmottled curly dwarf, and the leafhopper (*Euscelis exitiosus*) failed to transmit spindle tuber. Only a few tests were made in both the instances where negative results were obtained.

3. Nine collections of grasshoppers, four of tarnished plant bugs, and two of flea-beetles were found to have been carrying the virus of either spindle tuber or unmottled curly dwarf when collected.

4. Transmission tests with grasshoppers and the tarnished plant bug gave negative results with mild and rugose mosaic and with leaf roll. The flea-beetle (*Epitrix cucumeris*) also failed to transmit mild mosaic and leaf roll.

5. In general, the percentage of infection increased with the number of insects used for the inoculation and with the reinoculation (repeated transfers) of a small number of insects.

6. Neither disease was transmitted by inoculations with either the saliva or an extract from the stomach of grasshoppers that had been fed on infected plants.

7. Cross inoculations with both diseases between the Bliss Triumph and a number of other varieties did not cause any great modification of symptoms, the greatest variation occurring in necrotic symptoms of leaves and stems.

8. Interspecific inoculations between spindle-tuber and unmottled-curly-dwarf potatoes and a number of other solanaceous plants were all negative.

9. The virus of "masked" mosaic of apparently healthy plants is also carried in spindle-tuber and unmottled-curly-dwarf plants, as evidenced by the appearance of mosaic symptoms when cross-inoculations were made to *Datura stramonium, Nicandra physalodes,* and *Physalis heterophylla.* No mosaic symptoms occurred upon inoculation back to apparently healthy potatoes.

10. With artificial inoculations the percentage of infection was increased by increasing the amount of inoculum, and the rate of development of current-season or perpetuation symptoms was directly correlated with the time of inoculation.

11. Both diseases were transmitted by seed-piece contact and by the cutting knife, the diseases being carried to four successive seed pieces.

12. A treatment of the seed pieces with organic mercury was effective in preventing infection when applied immediately after inoculation but not six hours later. Holding the seed pieces for six hours after inoculation was not effective in preventing infection but holding for 15 to 24 hours greatly decreased the amount of infection.

13. More infection occurred when seed pieces inoculated by contact or by the cutting knife were immediately planted in a moist soil than when the soil was dry.

14. Tests which simulated the mechanical injury caused by picker planters indicated that such machines can transmit the disease but the amount of transmission would be negligible unless a high percentage of the seed tubers were infected.

15. The spindle-tuber virus did not spread or diffuse thru the tuber in storage as a result of seed-piece plug inoculations.

16. The spindle-tuber virus withstood a greater dilution than that of unmottled curly dwarf. Only a few questionable infections were obtained with dilutions greater than 1 to 1,000.

17. The thermal death point of the virus contained in the juice of unmottled-curly-dwarf plants was found to lie between 75° and 85° C.

Infection Experiments with Spindle Tuber and Unmottled Curly Dwarf of the Potato

R. W. GOSS[1]

INSECT TRANSMISSION

Numerous investigators have shown that aphids can transmit leaf roll and the various mosaics of potato, and Schultz and Folsom (18) have reported transmission of spindle tuber and unmottled curly dwarf by aphids. Investigations of the transmission of potato virus diseases by insects other than aphids have been somewhat contradictory. Positive evidence of insect transmission, if properly checked, necessarily outweighs inconclusive negative evidence, and as a result of investigations chiefly in foreign countries, other groups of insects have been found capable of transmitting certain virus diseases. The chief groups of insects involved have been capsids transmitting leaf roll (Murphy, 14) and mosaic (Elze, 2), leafhoppers or jassids (Cicadellidae) transmitting leaf roll (Murphy, 14, Elze, 2) and mosaic (Smith, 20), and flea-beetles transmitting leaf roll (Murphy, 14, Elze, 2). In addition, the transmission of mosaic by white fly was reported by Smith (20), and Elze (2) reports transmission of mosaic by *Mamestra brassicae* and of crinkle by leafhoppers or jassids. The only published reports of investigations of spindle tuber and unmottled curly dwarf dealing with insects other than aphids have been those of the author on transmission by grasshoppers (6) and a preliminary abstract of the present work (7).

Even though evidence contradictory to some of these results on leaf-roll and mosaic transmission have been reported by Schultz and Folsom (17, 18), Hungerford (9), and Smith (20), it is probable that more than one insect species are capable of spreading some of the potato virus diseases. This conclusion was also indicated by repeated observations in the dry-land section of western Nebraska. While aphids are abundant and are probably the chief vectors of potato virus diseases in the more humid sections of the country, they are usually scarce in the western upland plains area and are not present in large enough numbers to account for the rapid spread of spindle tuber and unmottled curly dwarf which sometimes occurs. It was therefore considered advisable to determine the common potato insects in this area and to test them as transmitting agents.

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[1] The author is indebted to R. W. Samson, Karl Koch, and Edgar Tullis for valuable assistance during the progress of these investigations.

Many phases of the studies herein reported require further investigation, but the discontinuance of the project has made it advisable to publish the results obtained up to date in the hope that they will be of value to other investigators in the field.

FIELD OBSERVATIONS

During the summers of five years, 1924 to 1928, surveys were made of potato fields in different parts of western Nebraska. The usual procedure was to count the number of each species of insect occurring on a certain number of plants in a field. In the experimental plots and near-by fields counts were made weekly for several years. In addition more general observations were made of the occurrence of insects in fields over larger areas. In discussing the distribution of insects in western Nebraska, it is well to remember that two different types of culture are practiced, the close-planted ridged rows under irrigation in the North Platte Valley with the resultant heavy foliage covering the ground by mid-season and the wider-spaced flat cultivation with smaller plants found on the table lands where most of the seed potatoes are produced. The following insects and their distribution and prevalence were noted.

APHIDS.—This insect was found to be more abundant in the irrigated than in the dry-land fields, but even under these more favorable conditions no heavy infestation was noted during the five years. In 1925 surveys were made at intervals of about 10 days in experimental potato plots under irrigation where no attempt had been made to control insects, and the heaviest infestation found was 1 to 7 aphids on 38 per cent of the plants. In the same plot aphids were not found in 1924 and 1926 and were not found in 1927 until September. In other fields examined in this irrigated section, the heaviest infestations varied from 1 to 5 per plant on 10 to 25 per cent of the plants. In the dry-land sections only one field was found heavily infested during the five years, and this field contained 5 to 10 aphids on about 30 per cent of the plants. In 1928 a survey of 50 to 75 dry-land fields failed to reveal any aphids.

GRASSHOPPERS (*Melanoplus* spp.).—These were found to be more prevalent throughout the whole western-Nebraska potato section than any other insect. Occasionally they occur in great numbers, particularly in irrigated potato fields in close proximity to alfalfa, dry roadsides, or unirrigated fields.

FLEA-BEETLES (*Epitrix cucumeris* Harr. and *Systena taeniata* Say).—Flea-beetles were present to a slight extent in most localities but were abundant enough to cause serious damage in only two sections, the irrigated fields of Scotts Bluff county, north of the North Platte river, and in the northern area around Belmont and Marsland. In these sec-

tions they occur in considerable numbers and are the worst insect pests on potatoes.

COLORADO POTATO BEETLES (*Leptinotarsa decemlineata* Say.)—This insect is usually of minor importance, and while it occurs commonly in many fields, it is not usually severe enough to warrant control measures. Only a few fields were found seriously invaded and it was more prevalent around gardens.

TARNISHED PLANT BUGS (*Lygus pratensis* L.).—These are usually of minor importance, occurring in small numbers in many fields. They were found quite abundant in 1928 in the same area in which flea-beetles occurred.

The gray and the striped blister-beetles (*Epicauta cinerea* Forst. and *E. vittata* Fab.), the black blister-beetle, (*Epicauta pennsylvanica* De G.), and the leafhopper (*Empoasca mali* Le Baron) are usually present in relatively small numbers and are of minor importance.

In summarizing the foregoing notes we can state that in dry-land fields insects are not usually abundant, that grasshoppers are the most widely distributed, and that flea-beetles are the most serious pests in certain local areas. In the irrigated fields these same two insects are most abundant, and in addition aphids may sometimes occur in large enough numbers to be of importance in the spread of virus diseases.

EXPERIMENTAL STUDIES

During the years 1925 to 1927 the experimental plots were located at the Scottsbluff substation and were irrigated. In 1928 the plot was located at Belmont in the dry-land area.

Methods.—The methods used in making the tests were essentially the same each year. The seed potatoes planted in the plots were all indexed in the greenhouse the preceding winter and only disease-free tubers were used. They were planted in the field in tuber-units and as soon as they emerged each plant was cut back to a single stalk. The infected plants used as the source of inoculum were usually grown in an adjacent plot. The plots were sprayed at intervals of about 10 days thruout the season with Bordeaux plus lead arsenate and nicotine sulfate.

The insects were obtained as far as possible from non-solanaceous plants. When they were collected from potatoes they were selected from healthy plants in fields having a low percentage of disease. During the first two years insect-proof cages were used to cover the plants being inoculated while the control plants were left uncaged. The very small amount of uncontrolled transmission which was found to

occur in these uncaged plants was thought to justify the elimination of the large cages. In the later years, therefore, small cages were used to cover single leaflets of a plant, and the youngest leaves were used for the inoculations.

Each insect collection was first placed on a healthy caged plant, with an uncaged plant in the same tuber-unit used as a control. This method was used to obtain a record of any virus-bearing insects at the time of collection. After having fed for 12 to 48 hours on this plant they were transferred to the caged, infected plant for an equal length of time and then transferred to a healthy plant. An uncaged healthy plant in the same tuber-unit was used as a control. After having fed for 24 to 48 hours on the plant being inoculated, the insects were killed and were then mounted for later species identification. These methods were used in all the tests included in Table 1 except with the grasshopper tests where in some instances the inoculations were repeated with the same insects. The number of insects used varied greatly in different tests. Even though the same number of insects was originally placed in a cage, some would die and others would not feed regularly. A record was kept of the number of actively feeding insects present through most of the feeding period in each inoculation. The number of grasshoppers varied from 1 to 15, Colorado potato beetles from 1 to 10, and the other insects from 8 to 25. The insect inoculations were made as soon after emergence as possible, most of the tests being made during the first three weeks of July.

A record was made of all symptoms appearing in the field, and at harvest each hill was dug and bagged separately and a record was made of all tuber symptoms. One tuber from each of these single-stalk hills was indexed in the greenhouse and the plants in the greenhouse were kept until the tubers developed. In all instances of positive or doubtful infection another seed piece from the same tuber and one from another of the same hill were grown in the field the following summer. With this method any partial infection of tubers or hills might easily have been missed. If all tubers from each hill had been planted the following year, the percentages of infection would probably have been found higher than those reported in Table 1, as this was true in other experiments. When healthy controls developed disease symptoms, the inoculation series was discarded and the results are not included in the table.

TABLE 1.—*Summary of insect inoculations* [1]*— 1925-1928*

Insect	Spindle tuber			Unmottled curly dwarf			Rugose mosaic		Mild mosaic		Leaf roll	
	Plants inoculated	Plants infected		Plants inoculated	Plants infected		Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	No.	*No.*	*P. ct.*	*No.*	*No.*	*P. ct.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Grasshoppers *Melanoplus spp.*	118	44	37.6	16	3	18.7	4	0	1	0	2	0
Flea-beetle *Epitrix cucumeris*	30	6	20.0	21	5	23.8			15	0	12	0
Flea-beetle *Systena taeniata*	14	1	7.1	11	5	45.5						
Leaf beetle *Disonycha triangularis*	12	4	33.3	4	0	0.0						
Tarnished plant bug *Lygus pratensis*	26	7	26.9	18	7	38.9	1	0	7	0	14	0
Colorado potato beetle *Leptinotarsa decemlineata*	24	5	20.8	17	1	5.9						
Leafhopper *Euscelis exitiosus*	3	0	0.0									

[1]All inoculations where the healthy control became infected were omitted from the table. The amount of infection in the healthy controls was less than 1 per cent. Only 4 of the 460 plants used as controls became infected.

Results.—A summary of the inoculations made during the four-year period, 1925 to 1928, is presented in Table 1.

The amount of uncontrolled transmission as determined by the infected control plants was very small and can be disregarded in the results reported, since only 4 of the 460 controls became infected.

Most of the insects tested were able to transmit spindle tuber and unmottled curly dwarf. The percentage of infection of each disease varied with different insects and the infection of the two diseases varied with the same species of insects. This was probably due to the wide variation in the number of tests conducted, and to the variable number of insects used in different tests. The fact that infection occurred in many instances in repeated tests for several years is considered of more importance than the actual percentage of infection. As already stated, all the percentages of infection would probably have been higher if it had been possible to plant all of the progeny of each plant. Current-season symptoms seldom appeared except in the tubers.

Spindle tuber was transmitted by grasshoppers (*Melanoplus* spp.); the flea-beetles (*Epitrix cucumeris* and *Systena taeniata*); the leaf beetle (*Disonycha triangularis*); the tarnished plant bug (*Lygus pratensis*); and the larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*). Negative results were obtained with the leafhopper (*Euscalis exitorus*). Unmottled curly dwarf was transmitted by all of the same species that transmitted spindle tuber except *Disonycha triangularis*. The two instances of negative results with these diseases are rather doubtful because the number of inoculations with these two insects was small.

It was found that in some instances the insects were carrying the virus of spindle tuber when they were collected. As previously stated, each collection of insects used for an inoculation was fed on a healthy plant before being used in the test. In these tests, nine grasshopper, four tarnished plant bug and two flea-beetle collections were found carrying the virus of spindle tuber or unmottled curly dwarf when collected.

Transmission tests with grasshoppers and the tarnished plant bug gave negative results with rugose and mild mosaic and with leaf roll. *Epitrix cucumeris* also gave negative results with mild mosaic and leaf roll.

While only a few insects were tested in the present investigations for mosaic and leaf-roll transmission, and in some instances the number of inoculations was too small to be reliable, it was clearly evident that grasshoppers, flea-beetles, and tarnished plant bugs transmitted spindle tuber and un-

mottled curly dwarf under conditions that yielded negative results with mosaic and leaf roll. It is possible that some of these insects might be of use as a means of separating certain virus mixtures occurring in potato plants.

FACTORS INFLUENCING INSECT TRANSMISSION

Schultz and Folsom (18) found in their transmission tests with mosaic and leaf roll that in general the percentage of infection increased with the number of aphids, although they obtained some conflicting evidence. Smith (21), using 2, 6, 12, and 18 aphids, also obtained the greatest incidence of leaf-roll infection with the largest number of aphids. In many reported investigations of insect transmission the insects were kept in a cage containing both a healthy and an infected plant. The chances for infection under such conditions would be greater than in the author's experiments where the insects were fed successively upon infected and healthy plants for only 12 to 48 hours. It was thought that this might account for the low percentage of infection obtained with some of the insects tested and experiments were made to determine the effect of the number of insects used in the inoculation.

These tests were made by using varying numbers of insects in single inoculations and by using the same insects for repeated inoculation of the same plant. Some of the results obtained with grasshoppers on spindle-tuber transmission have been reported (6). Further tests with grasshoppers confirmed the results. A few tests with other insects yielded somewhat similar results with spindle tuber. Two single inoculations with *Lygus pratensis,* using 12 insects, were negative while four triple inoculations with the same number resulted in three infections. Five single inoculations with the larvae of the Colorado potato beetle, using three insects, were negative while four double inoculations with the same number resulted in two infections. In further tests with grasshoppers it was found that a single inoculation of a larger number (8 to 15) of insects gave about the same amount of infection as a smaller number with repeated inoculation.

This may have been due to accidental factors of feeding rather than to the amount of inoculum carried by the insects. With small numbers of inoculations the results were quite variable even with the same number of insects. This was particularly true with unmottled-curly-dwarf inoculations. None of the insects tested, including aphids, appeared to feed with any relish upon the infected plants, which may be one

explanation of the relatively small amount of this disease occurring in commercial fields as compared with spindle tuber.

The time interval occurring between feeding on an infected and a healthy plant might be a factor in determining infection. One set of inoculations with spindle tuber and unmottled curly dwarf using 6 to 15 grasshoppers with time intervals of 1, 6, and 12 hours and 1, 2, 4, 6, 8, and 10 days, during which periods of time the insects were held in glass vials, resulted in only one infection with spindle tuber with a six-day interval. Further tests would be desirable to determine this point, as the number of inoculations was evidently too small for definite results.

A few tests were made with spindle tuber, using grasshoppers as the transmitting agents, to determine whether the insects could transmit the disease to more than one plant after feeding on an infected plant. In this single test the following results were obtained: when grasshoppers after feeding on a spindle-tuber plant were held for one day each on two successive plants, no infection resulted; on three plants, no infection; on four plants, the second and third plant were infected; on five plants, no infection; on six plants, the fourth plant was infected. The results indicate that grasshoppers in transmitting the spindle-tuber virus may transmit the disease to a number of plants.

The fairly high percentages of spindle-tuber infection obtained by the use of grasshoppers as transmitting agents and the marked difference in their feeding habits as contrasted with aphids seemed to offer an opportunity to study the methods by which the insect carried the virus. A few preliminary tests were made using the saliva secreted by grasshoppers and an extraction of the stomach.

In the saliva test, 35 grasshoppers were fed upon spindle-tuber plants and an equal number on unmottled-curly-dwarf plants. They were then forced to secrete saliva into clean glass vials. The saliva was diluted with an equal amount of distilled water and inoculated into healthy plants by making incisions in the stem at the base of the three youngest petioles with a scalpel dipped in the inoculum. A total of 36 inoculations were made, some being made immediately and others after intervals of 1, 2, 4, and 5 hours. No infection resulted.

Fifteen grasshoppers were fed upon unmottled-curly-dwarf plants for two days. The stomachs were then removed and ground in a mortar with 5 c.c. of distilled water. Six inoculations were made, similar to those in the saliva tests. No infection occurred.

Either the virus is not carried in the saliva or the stomach in sufficient quantities to cause infection when treated in this manner or else the method of inoculation was not satisfactory.

More detailed and extensive experiments are needed to determine the effect of the many factors that may enter into the successful transmission of virus diseases by insects. In most experiments, as in those reported here, the actual feeding of the individual insect has not been observed in detail and until this is done we cannot clearly understand either the mechanics of transmission or the possible biological relations between insect and virus. The effect of the environment upon the virus as it is carried by the insect in the intervals between feeding on diseased and healthy plants and the effect of the age of the host plant and its composition as influenced by environment upon the feeding habits of the insect vector and the infection of the plant with the virus are also dependent upon such detailed investigations.

ARTIFICIAL INFECTION EXPERIMENTS

INOCULATION METHODS

Numerous methods have been used by different investigators to artificially introduce the infectious matter from a virus-infected plant into a healthy one. Schultz and Folsom (18) reported the transmission of spindle tuber and unmottled curly dwarf by grafts and leaf mutilation. In the author's investigations a method was desired for inoculating fairly large numbers of plants under field conditions. Preliminary tests were made with a number of different methods and the results are shown in Table 2.

It was evident from these preliminary tests that both diseases could be transmitted in a variety of ways but that

TABLE 2.—*Preliminary tests of inoculation methods*

Method of inoculation[1]	Spindle tuber		Unmottled curly dwarf	
	Inoculated	Infected	Inoculated	Infected
	Number	*Number*	*Number*	*Number*
1. Hypodermic injection	6	0	..	..
2. Stem grafts	6	1	..	..
3. Needle prick, 50 punctures per plant	30	3	24[3]	5[3]
4. Leaf mutilation	9	5	13[3]	12[3]
5. Insertion of wedge of tissue into stem	11	6	8	8
6. Insertion of crushed leaves into stem	7	7	5	5
7. Three insertions of cotton saturated in extract into stem[2]	7	7	4	4
8. Seed-piece inoculation with core plugs[2]	46	41	..	..

[1] All of the inoculations were made on plants grown in tuber units. All the control plants remained healthy.

[2] Methods 7 and 8 were adopted for most of the field experiments reported in this publication.

[3] The inoculated plants were held under glass jars for 36 hours after inoculation.

some methods were successful more often than others. The only negative results were obtained with hypodermic injections. Stem grafts were not successful, owing to the difficulty of obtaining a good union under dry field conditions. The needle-prick method, using a small piece of cotton saturated with extract on the tip of a needle and making about 50 punctures per plant, resulted in a small percentage of infection, possibly due to the rapid drying of the drop of extract. When plants inoculated in this way with unmottled curly dwarf were held under a bell-jar for 36 hours, a higher percentage of infection was obtained. Leaf-mutilation inoculations were fairly successful but repeated experiments produced quite variable results, possibly due to the rapid drying of the injured leaves, as indicated by the higher percentage of infection obtained with unmottled curly dwarf when the inoculated plants were held under a bell jar for 36 hours. The necessity of covering inoculated plants with a bell jar in order to obtain a high percentage of infection made both the leaf mutilation and needle-prick methods unsuitable for field experiments.

Methods 5 and 6 (Table 2) consisted of inserting infected tissue into a slit cut in the stem of a healthy plant and then wrapping with damp cotton and waxed paper. These inoculations resulted in a very high percentage of infection but the methods involved considerable time and labor and were unsuited for field experiments. The method was therefore modified by inserting a piece of cotton saturated with the extract of infected plants into small wounds cut with a scalpel in the stem at the axils of the three youngest petioles. This method resulted in 100 per cent infection in the preliminary tests (Method 7, Table 2) and was therefore adopted for further experimental work and was used in all juice inoculations of foliage hereafter reported unless otherwise specified.

In all the methods previously mentioned it was found that current-season symptoms seldom developed in the tops and that in many instances symptoms were lacking in the tubers, thus making it necessary to index all plants in the greenhouse or replant the following year in order to determine the results of the inoculation. It was thought that if the seed piece could be inoculated at the time of planting there would be a better chance of obtaining current-season symptoms. Such a method would also obviate the necessity of making repeated long-distance trips to isolated experimental plots in order to make juice inoculations of the foliage at the proper stage of growth. The method developed was that described by the author in a note published in 1926 (4) and was some-

what similar to a method described at about the same time by Murphy (15). The method consists of the insertion of a core of tissue, obtained from an infected tuber with a cork borer, into a hole in a healthy seed piece made with another cork borer one size smaller. The ends of the plug are cut off to avoid the development of sprouts from eyes occurring on the plug. The use of the smaller-size hole insures a firm contact and the cylindrical shape provides a relatively large surface. A high percentage of infection resulted from this method of inoculation (Method 8, Table 2). A large percentage of the inoculated plants produced tubers with well developed symptoms and following the unmottled-curly-dwarf inoculations the foliage symptoms were distinct two weeks after emergence. This method was adopted for many field experiments and will be referred to as seed-piece core or plug inoculations.

After the preliminary tests in which the seed-piece plug inoculations were found to be successful a further test was made to determine the relative infectiousness of plugs obtained from different portions of infected tubers placed in different portions of healthy tubers. Plugs from infected tubers, divided into pieces 5 mm. long, showed no difference in infective power between the outer and inner portions. No differences occurred with plugs taken from different distances from the eyes of infected tubers or from apical and basal ends and placed in different portions of healthy tubers. Four infected plugs placed around one eye of a healthy seed piece produced symptoms similar to those caused by one plug.

It was found that combination diseases when inoculated into a healthy seed piece by this core-plug method often split up in much the same manner as occurs with juice or aphid inoculations. Three inoculations made from tubers affected with rugose mosaic and spindle tuber resulted in one rugose mosaic, one spindle tuber and one healthy plant. Six inoculations with a combination of mild mosaic and spindle tuber resulted in two mild mosaic, two mild mosaic combined with spindle tuber, one spindle-tuber and one healthy plant. Three inoculations of a leaf-roll spindle-tuber combination resulted in two leaf-roll and one healthy plant. It was also found that it was possible to inoculate a healthy seed piece with two diseases by using two plugs, one from each of the infected tubers, the inoculated plant showing the symptom complex of both diseases.

INTERVARIETAL INOCULATIONS

A number of intervarietal inoculations were made to determine the possiblity of modification of symptoms and to deter-

mine whether the two diseases could be identified by distinguishable symptoms in other varieties.

In 1926, inoculations were made in the field by the insertion of a wedge of tissue cut from an infected stem into a slit cut in a healthy stem and then bound with damp cotton and waxed paper. Thirty inoculations were made from spindle-tuber and unmottled-curly-dwarf Green Mountains and Triumphs to each of these varieties. While there was some slight modification, the essential differences in the symptoms of the two diseases were evident in the inoculated plants regardless of the variety used as the source of inoculation. The unmottled-curly-dwarf Green Mountain tubers were obtained from Schultz and Folsom, while the infected Bliss Triumph tubers were from Nebraska collections. The unmottled curly dwarf was apparently the same in both varieties, as judged by the symptoms produced in Bliss Triumph.

In 1927, inoculations of spindle tuber and unmottled curly dwarf were made from Bliss Triumph and Green Mountain to both varieties. The inoculations were made at planting, using the seed-piece plug method. The plants were grown in an irrigated field. The tuber used as the source of inoculum, and the inoculated seed pieces with the healthy controls from the same tubers, were planted in units in the row.

The results of these tests are presented in Table 3. Spindle tuber was transmitted from Green Mountain and Bliss Triumph to both varieties. The inoculation of Bliss Triumph from both varieties resulted in a higher percentage of infection than when Green Mountain was inoculated. The disease caused a much greater reduction in yield with Bliss Triumph than with Green Mountain and this reduction was evident as a current-season effect in the inoculated plants. The disease did not cause any reduction in the number of tubers produced. The symptoms of the disease were evident by the middle of July on all the plants infected as a result of the seed-piece inoculation. Ruffling and erectness were equally distinct in both varieties. Dwarfing was very slight in the inoculated plants as compared with the plants used as a source of infection. The tuber symptoms were the same in both varieties and were as severe in the inoculated plants as in those used as a source of inoculum. The chief symptoms were the increase in number and decrease in the shallowness of the eyes, an increased length in proportion to width, and a decrease in color in the Bliss Triumph. A slght amount of cracking was also present in the Bliss Triumph tubers.

TABLE 3.—*Intervarietal transmission of spindle tuber and unmottled curly dwarf by seed-piece inoculations* [1]

Source of inoculum	Variety inoculated	Healthy controls	Number of plants	Infected plants[2]		Tubers per hill[3]	
						Number	Weight
				Number	*Per cent*	*Number*	*Grams*
SPINDLE TUBER							
Green Mountain			5	5	100	8	904
Bliss Triumph			11	11	100	9	733
Green Mountain	Green Mountain		7	1	14	11	1010
Green Mountain	Bliss Triumph		4	3	75	5	260
Bliss Triumph	Bliss Triumph		22	15	68	7	704
Bliss Triumph	Green Mountain		4	1	25	6	1100
		Bliss Triumph	11	0	0	6	1034
		Green Mountain	5	0	0	6	1312
UNMOTTLED CURLY DWARF							
Green Mountain			2	2	100	4	257
Bliss Triumph			27	27	100	4	100
Bliss Triumph	Bliss Triumph		62	16	25	7	247
Bliss Triumph	Green Mountain		14	8	57	10	566
Green Mountain	Green Mountain		2	0	0		
Green Mountain	Bliss Triumph		2	0	0		
		Bliss Triumph	23	0	0	7	1284
		Green Mountain	6	0	0	6	1282

[1]The seed pieces were inoculated by the core-plug method.

[2]The percentage of infection is based only on current-season symptoms.

[3]The number and weight of tubers per hill are determined from the infected hills except with the healthy controls.

Unmottled curly dwarf was transmitted from Bliss Triumph to both varieties. The percentage of infection was higher with the inoculation of Green Mountain. The few tests using Green Mountain as a source of inoculum were negative. The unmottled-curly-dwarf Triumph plants used as a source of inoculum were greatly retarded in emergence, but no effect on emergence was noticeable with the inoculated plants. There was a great reduction in yield of the inoculated plants, but the yield was even lower with the plants used as a source of inoculum. There was no decrease in set of tubers resulting from inoculation, but the set was reduced in the plants used as a source of inoculum. The symptoms on all infected plants were evident by the middle of July, altho the dwarfing was greater in the plants used as a source of inoculum than in the inoculated plants. The tuber symptoms were about the same with both varieties. About 40 per cent of the infected tubers were severely cracked, the cracking being as severe in the tubers from inoculated plants as in those from the source of inoculum.

An additional symptom of unmottled curly dwarf was noted for the first time in this experiment as a necrosis occurring both internally and on the surface of the tubers. This symptom has been described by the author (8). About 30 per cent of the tubers from the plants used as a source of inoculum and 80 per cent of the tubers from inoculated plants showed surface necrosis. This necrotic condition appeared first around the lenticels. The spots enlarged, grew together, and sometimes more than half the tuber was affected. Internal necrosis was also most severe in tubers from inoculated plants and occurred in varying degrees of severity from a light brown flecking and streaking in the vascular region to a severe necrosis partially affecting all tissues except the internal pith cells.

During the winter of 1925 and 1926, a considerable number of seed-piece inoculations were made between the varieties Bliss Triumph and Green Mountain, Irish Cobbler, Early Ohio, White Rose, British Queen and Downing. The infected tubers of the different varieties were obtained from many sources and were all labeled by the senders as spindle tuber, altho many of the plants grown in the greenhouse from these tubers appeared to be unmottled curly dwarf. Due to the incomplete history of the seed and the fact that many tubers had been selected in open fields where they were subject to mixed infection, it was difficult in this one test to accurately distinguish between spindle tuber and unmottled curly dwarf in the different varieties.

A high percentage of infection with either spindle tuber or unmottled curly dwarf was obtained with all cross inoculations. Cross inoculations of spindle tuber between Irish Cobbler, Early Ohio, and Triumph, and from Triumph to Downing did not cause any modification of symptoms. Spindle-tuber inoculations from Triumph to British Queen resulted in much more severe symptoms. Inoculations from Downing to Triumph and cross inoculations between Green Mountain and Triumph produced variable results, possibly due to mixed infection in the plants used as the sources of inoculum. The only constant change in symptoms in the inoculations between the Triumph and the Green Mountain and Downing varieties was the absence of necrotic symptoms on the Triumph variety, while the Downing and Green Mountain plants showed some streaking, spotting, and burning.

INTERSPECIFIC INOCULATIONS

Attempts were made in 1927 and 1928 to determine whether certain solanaceous plants could carry the virus of spindle tuber and unmottled curly dwarf and possibly serve as overwintering hosts or as additional sources of infection in the field.

During the winter of 1927 ten tomato plants were inoculated with the juice obtained from spindle-tuber leaves by the needle-prick method. Eight additional tomato plants were inoculated similarly with juice from healthy potato plants as controls. The inoculated plants were held in a moist chamber for 24 hours. Six weeks after inoculation, juice obtained from the tomato plants was inoculated into five healthy potato plants.

All the tomatoes developed mosaic. When inoculated back to potatoes, neither mosaic nor spindle tuber developed and the progeny remained healthy.

In another test, nine tomatoes were inoculated in a similar manner from spindle-tuber potatoes. All inoculated plants developed mosaic, while the uninoculated controls remained healthy. Macerated tissue and juice of the tomato plants were inserted into holes bored in 24 healthy potato seed pieces. None of the resulting plants or their progeny developed either mosaic or spindle tuber.

In 1928, more extensive tests were made with additional hosts, using both insect and juice inoculations. The tests were conducted in the greenhouse. The aphid *Myzus persicae* Sulz. was colonized in two cages, one containing spindle-tuber potato plants and the other unmottled-curly-dwarf. The solanaceous plants to be tested were grown in small pots.

Five to ten aphids were transferred from the source of inoculum to the plant to be tested and kept under lamp chimneys closed with cheesecloth for two days, after which the aphids were killed. Two weeks later aphids from a healthy stock were fed on these plants and then transferred to healthy potatoes, and juice inoculations were also made to young potato plants.

The plants used in this test were Jimson weed (*Datura stramonium* L.), Buffalo bur (*Solanum rostratum* Dunal.), ground cherry (*Physalis heterophylla* Nees), Apple of Peru (*Nicandra physalòdes* (L.) Pers.), and tomato (*Solanum lycopersicum* Mill.).

Parallel inoculations were made by juice transfers. The leaves of an infected plant were crushed in a mortar. The two youngest leaves of the plant to be inoculated were punctured 20 times each with a fine needle covered with a wad of cotton saturated in the juice.

From 10 to 20 inoculations were made with each host. None of the inoculations from these hosts to young, healthy potato plants resulted in the appearance of either spindle-tuber or unmottled-curly-dwarf symptoms in either tops or tubers.

Most of the inoculations, both insect and juice, to *Datura stramonium* resulted in distinct mottling with some wrinkling and rugosity. Mosaic symptoms on *Nicondra physalòdes* were accompanied by necrotic areas on leaves and some leaf dropping. No necrosis developed on ground cherry, but distinct mottling and dwarfing occurred. This appearance of mosaic by inoculation from spindle-tuber and unmottled-curly-dwarf plants was similar to that reported by other workers as occurring particularly with Jimson weed following inoculation with the juice of an apparently healthy potato plant. It is apparent that this masked mosaic also occurs in plants infected with spindle tuber and unmottled curly dwarf. When inoculations were made back to apparently healthy potatoes from these mottled plants no symptoms appeared in the potato plant.

These plants, judged by the small number of tests here reported, are not capable of carrying the virus of spindle tuber and unmottled curly dwarf, but do develop mosaic symptoms when inoculated from potato plants which have no apparent mosaic symptoms.

THE EFFECT OF THE AMOUNT OF INOCULUM AND THE TIME OF INOCULATION UPON INFECTION

In various inoculation experiments in which Method 7 (the insertion of cotton saturated in extract into the stem of a

healthy plant) was used, considerable variation occurred in the percentage of infection and the development of the symptoms. This variation in results may have been caused by a number of factors, several of which could be subjected to experimental test. Experiments were therefore planned to test first the effect of the amount of inoculum and second the effect of the time of inoculation in relation both to the age of the plant inoculated and to the environmental conditions existing at the time of inoculation upon the percentage of infection and the development of symptoms.

The effect of the amount of inoculum.—In this test five-hill units of healthy plants were used, one hill for each of four inoculations (Table 4) and one left as a control. The inoculations were made when the plants were about six inches tall with juice obtained from young spindle-tuber and unmottled-curly-dwarf plants of known history. The inoculations were made by either one, two, or three insertions of cotton, saturated in the juice of an infected plant, into slits in the stem at the axil of the youngest petiole or the youngest two or three petioles, depending upon the number of insertions.

TABLE 4.—*The effect of the amount of inoculum upon the percentage of infection*

Treatment[1]	Plants inoculated	Plants infected		Plants inoculated	Plants infected	
	No.	*No.*	*P. ct.*	*No.*	*No.*	*P. ct.*
	SPINDLE TUBER			UNMOTTLED CURLY DWARF		
1. Single inoculation	20	8	40	18	1	5
2. Double inoculation	20	10	50	17	2	12
3. Triple inoculation	19	12	63	16	5	31
4. Triple inoculation (remoistened)	19	11	58	20	6	30
5. Control	20	2	10	20	2	10

[1]Inoculations were made by Method 7 (see page 13 and Table 2) with the triple inoculation remoistened. The cotton inserted into the stem was remoistened with inoculum one hour after the inoculation.

The data on the infected plants presented in Table 4 include all symptoms occurring either in the field or in the indexed plants in the greenhouse. Each hill in the test was a single-stalk hill, dug and bagged separately, and one tuber from each hill was indexed in the greenhouse.

With the spindle-tuber inoculations the results do not indicate any great difference in the percentages of infection.

There was no difference in the time of appearance of the symptoms with the different methods of inoculation. About 25 per cent of the infected plants developed current-season tuber symptoms and most of the others produced foliage symptoms when indexed in the greenhouse. The percentage of infection was greater with spindle tuber than with unmottled curly dwarf and none of the plants infected with unmottled curly dwarf developed current-season symptoms. The percentage of infection was very low with the single and double inoculations, whereas the triple inoculations resulted in a decidedly higher percentage of infection.

The relation of the time of inoculation to the amount of infection and the development of the symptoms.—This experiment was conducted under dry-land conditions in western Nebraska in 1928. Spindle-tuber and unmottled-curly-dwarf seed-tubers of known history were used for seed-piece inoculations by the plugging method at planting time. The remainder of the infected seed was planted and the juice obtained from the foliage of these plants on two different dates three weeks apart, was used to inoculate healthy plants grown from other seed pieces of the same healthy tubers used for the seed-piece inoculations. The fourth seed piece from the healthy tubers was planted as a control.

The seed-piece inoculations were made at the time of planting, June 8. The first juice inoculations were made when the plants were about six inches tall on July 16 and the second juice inoculation on August 6, when the plants were all about 14 inches tall. The juice inoculations were made by three insertions of cotton, saturated in the juice of infected plants, into the stem and remoistened with the inoculum after one hour, as in Inoculation 4, Table 4.

The plants were examined for symptoms on July 18 and 29 and August 8. No examination for foliage symptoms was made on the second set of juice inoculations. Each hill was dug and bagged separately and all tubers were examined for symptoms. One tuber from each plant was indexed in the greenhouse and both the tops and tubers of the indexed plants were examined for symptoms.

The results are presented in Table 5. All of the plants grown from the infected tubers used as the source of inoculum showed typical symptoms of the respective diseases. Four of the healthy controls in the spindle-tuber set became infected. None of these showed any foliage symptoms and only one produced tubers with spindle-tuber symptoms, so mine the possibility of modification of symptoms and to deter-

the field due to the close proximity of the infected plants. Only one control plant in the unmottled-curly-dwarf set became infected.

TABLE 5.—*The relation of the time of inoculation to the amount of infection and the development of symptoms*

Row	Treatment	Plants inoculated	Plants infected		Time of first appearance of symptoms in infected plants			
					Current-season		Greenhouse	
					Foliage	Tubers	Foliage	Tubers
		No.	*No.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
	SPINDLE TUBER							
1.	Source of inoculum	25	25	100.0	100.0	....	....	....
2.	Seed piece inoculation, June 7	22	17	77.2	58.8	17.6	0.0	23.4
3.	Foliage inoculation, July 16	20	13	65.0	0.0	38.5	23.0	38.5
4.	Foliage inoculation, August 6	20	19	95.0	.....	5.2	15.8	79.0
5.	Healthy check	23	4	17.4	0.0	25.0	25.0	50.0
	UNMOTTLED CURLY DWARF							
6.	Source of inoculum	25	25	100.0	100.0	....	....	....
7.	Seed piece inoculation, June 7	18	5	27.8	60.0	20.0	20.0	0.0
8.	Foliage inoculation, July 16	21	10	47.6	0.0	30.0	60.0	10.0
9.	Foliage inoculation, August 6	21	17	81.0	.....	11.8	88.2	0.0
10.	Healthy check	22	1	4.5	0.0	0 0	0.0	100.0

A higher percentage of infection was obtained with spindle tuber than with unmottled curly dwarf in each set of inoculations. The highest percentage of infection with each disease unexpectedly developed from the last juice inoculations. On the basis of other experiments, the greatest amount of infection was expected to occur from the first set of juice inoculations made when the plants were about six inches high. It is probable that the weather conditions occurring at the time of inoculation influenced the percentage of infection. On July 10, the time of the first juice inoculation, the maximum, minimum, and mean temperatures were respectively 92°, 64°, and 78°, while on August 6, when the second juice inoculation was made, they were 86°, 52°, and 69° F.

In many other experiments a much lower percentage of infection has been obtained in the field in western Nebraska than in the greenhouse, probably because of high temperatures and low humidities.

The percentage of infected plants which developed current-season symptoms was directly correlated with the time of inoculation, as shown in Table 5. The percentage of infected plants developing current-season symptoms as a result of seed-piece inoculation was about 75 to 80 and from the first

juice inoculation the percentage was 30 to 40. From the second juice inoculation only 5 per cent of the spindle-tuber and 11 per cent of the unmottled-curly-dwarf plants produced tubers with distinctive symptoms. No foliage symptoms occurred with either set of juice inoculations.

INOCULATION OF SEED PIECES

Experiments conducted by the author in 1925 (5) showed that spindle tuber can be transmitted by cutting knives and by contact of freshly cut seed pieces. These results have been confirmed by Bonde (1), Kotila (11) and McCall (12). This type of transmission is of great importance in experimental work and in seed plots where the tubers are often planted in units immediately after they are cut by hand or by a tuber-unit planter. In commercial planting by the usual methods, however, the seed pieces often stand some hours before planting and the amount of transmission occurring may well be affected by the exposure of the cut seed pieces either before or after inoculation. It was also considered desirable to know whether the disease could be transmitted from an infected seed piece to a successive number of healthy seed pieces and whether the amount of transmission could be reduced by physical or chemical treatment of the seed pieces. The following tests were therefore made in 1926 to obtain information on these two points. For these experiments the spindle-tuber seed-tubers were selected from a field seriously infected with the disease and possibly containing some unmottled curly dwarf. The healthy seed was from a tuber-indexed lot.

Transmission to successive seed pieces.—In this test plugs of tissue were removed with a cork borer from infected tubers. Similar plugs were taken from healthy seed pieces. The core of tissue from the infected tuber was then passed thru the hole in the healthy seed piece and this was repeated with the same plug in four successive tubers, the plug being left in the fourth seed piece. Twenty-five plants were used for each test and there was no great variation in the percentages of infection following the successive inoculations, the results showing 26, 14, 33, and 18 per cent respectively for the four successive inoculations with the same core of spindle tuber. These percentages were all lower than those obtained in a comparable test of 25 hills in an adjoining row where 60 per cent infection was obtained by inserting a spindle-tuber core into each healthy seed piece. Another test with the same source of inoculum, in which the

spindle-tuber cores were washed in running water for one minute before being introduced into the healthy seed pieces, resulted in 64 per cent infection. It is probable that the higher percentage of infection obtained when the cores of infected tissue were left in the healthy seed piece was due to organic union. The results show that the disease may be transferred to a number of seed pieces from the infected tissue by contact and that the inoculum is not easily removed from the surface of the infected seed piece.

TABLE 6.—*The effect of different treatments after the inoculation of seed pieces by contact*

No.	Treatment	Plants	Infected plants	
		Number	*Number*	*Per cent*
1.	Spindle-tuber control	13	13	100
2.	Healthy control	32	0	0
3.	Contact inoculation, immediate planting	15	7	46
4.	Contact inoculation, immediate treatment[1] and planting	16	0	0
5.	Contact inoculation, treated[1] after 5½ to 6 hours	13	4	30
6.	Contact inoculation 5½ to 6 hours after cutting	7	1	14
7.	Contact inoculation; spindle tuber freshly cut, healthy cut 5½ to 6 hours	6	4	66

[1]Treatment was made with DuPont No. 12 Bel., 1: 10 dilution, as an instantaneous dip.

Treatment of inoculated seed pieces.—The results of this experiment conducted in 1926 are presented in Table 6, which lists the various methods used for inoculation and treatment. In three of the tests the seed pieces were inoculated immediately after cutting and treated in various ways. One of these was planted immediately. Another was treated with an organic mercury treatment immediately after being inoculated. A third was treated in the same way about six hours after the inoculation. Two other lots were inoculated and planted without any treatment, but one of these was allowed to stand for six hours after cutting before being inoculated, and, with the other the healthy seed, was allowed to stand six hours after cutting before being inoculated by contact with freshly cut spindle-tuber seed. In all tests where the cut seed was held for 5½ to 6 hours the seed pieces were kept in open paper sacks. The seed pieces for each treatment were planted in adjacent rows and one seed piece from each healthy and infected tuber was planted in a separate row as controls.

Unfortunately a severe storm washed out many of the seed pieces at about the time they were emerging. Only those which had developed a good root system were saved. As a result only about one-third of the seed planted could be used in determining the results, which accounts for the small and uneven number of plants listed in Table 6. Originally 50

seed pieces were used for each treatment, except Treatments 6 and 7 (Table 6), which contained 25 seed pieces each.

Even with the relatively small number of plants available, there are certain outstanding differences evident from the data presented in Table 6. All of the infected seed produced typical spindle-tuber plants and all of the healthy seed produced healthy plants. The immediate contact inoculation and planting resulted in 46 per cent infection as contrasted with the complete lack of infection obtained by inoculations made in a similar manner but treated with an organic mercury compound immediately after inoculation. When this treatment was delayed six hours, 30 per cent infection occurred. When the inoculation was made six hours after cutting and without treatment, only 14 per cent infection occurred, as contrasted with 66 per cent infection obtained when healthy seed was allowed to stand six hours and then inoculated with freshly cut spindle-tuber seed. The percentage of infection was even higher than when both seed pieces were freshly cut, the difference undoubtedly being due to the larger error with the smaller number of plants.

We can conclude that the treating of freshly cut seed with an organic mercury treatment is effective only when the treatment is made immediately after the seed pieces have been inoculated by contact with freshly cut infected seed pieces. A great reduction in the percentage of infection was obtained when the spindle-tuber seed was allowed to stand six hours after cutting and before contact. The reduction in the percentage of infection obtained by holding the inoculated seed six hours before treating may have been due partly to the length of the exposure rather than to the treatment, as no comparable data were obtained on this point. It was evident, however, that holding seed for six hours after inoculation was not an effective method of control.

Kotila in 1927 (11) reported tests of an organic mercury treatment of the inoculated seed pieces and a treatment of the cutting knife with mercuric cyanide. His results were negative because of a lack of transmission in the untreated controls which he attributed to holding the seed over night after inoculation before planting. McCall (12) reports variable results from treating the inoculated seed pieces with acidulated mercuric chloride for five minutes. He obtained a 39 per cent reduction of transmission with the Triumph variety, but no reduction in duplicate tests with the Irish Cobbler.

Effect of drying of seed pieces preceding inoculation.—Tests were conducted in 1927 to determine the effect of the drying of the cut surface of healthy seed pieces upon the percentage

of infection when juice obtained by scraping the freshly cut surface of an infected tuber was diluted with an equal amount of distilled water. The seed piece to be inoculated was dipped in two cubic centimeters of the diluted juice placed in a Petri dish. The inoculated seed pieces were not planted for 18 to 24 hours due to unfavorable weather conditions.

Twenty-five hills of unmottled curly dwarf and 21 hills of spindle tuber were grown from the tubers used as the source of inoculum. All of the tubers produced infected plants with the typical symptoms of the respective diseases.

Twenty-five inoculations with unmottled curly dwarf and 21 inoculations with spindle tuber were made immediately after cutting the healthy seed pieces. A similar number of inoculations were made with each disease 30 minutes, 1, 3, and 18 hours after cutting.

Only two infections were obtained, one with unmottled curly dwarf after a 30-minute interval and one with spindle tuber after a three-hour interval. The lack of infection as indicated by the absence of symptoms in both tops and tubers was probably due to the length of time, 18 to 24 hours, during which the inoculated tubers were held before planting.

Effect of delayed planting.—As a result of this experiment another test was made in 1928 to deterimne the effect of delayed planting after contact inoculation of seed pieces. As previously noted, Kotila (11) attributed the lack of infection in some of his experiments to holding the inoculated seed over night; and Schultz and Folsom (19) report that Bonde failed to secure infection when the inoculated seed pieces were allowed to dry before planting.

In this test healthy tubers were quartered and one seed piece was planted as a control on the other three, which were inoculated immediately after cutting by contact with the freshly cut surface of an infected tuber. One of these seed pieces was planted immediately and the other two were held for six hours and 15 hours in open paper sacks before planting. All four seed pieces from each tuber and the infected tuber used as a source of inoculum were planted in the same numbered hill of parallel rows as indicated in Table 7. Fifty inoculations were made with each disease for each treatment. The data presented in the table are based upon the number of hills which produced tubers. Each hill was harvested separately and notes made of all tuber symptoms. One tuber from each hill was then indexed in the greenhouse.

The results were rather disappointing, owing to the low percentage of infection obtained by contact inoculation and

TABLE 7.—*Effect of delayed planting after contact inoculation of healthy seed pieces with spindle tuber and unmottled curly dwarf*

Row	Treatment	Spindle tuber			Unmottled curly dwarf			
		Hills	Infected[1]	Hills	Row	Hills	Infected[1]	Hills
		No.	*No.*	*P. ct.*		*No.*	*No.*	*P. ct.*
1.	Source of inoculum	38	38	100.0	6	44	44	100.0
2.	Planted immediately	47	10	21.3	7	49	3	6.1
3.	Planted after 6 hours	41	8	19.5	8	44	3	6.8
4.	Planted after 15 hours	45	4	8.9	9	42	2	4.8
5.	Healthy checks	50	5	10.0	10	41	0	0.0

[1]The number infected included both those with tuber symptoms and those that developed symptoms when indexed in the greenhouse.

immediate planting in comparison with previous experiments. Five plants or 10 per cent of the healthy controls used in the spindle-tuber test were found to be infected, probably due to current-season insect transmission, as only two of the other 15 seed pieces from the same five tubers were infected. The amount of infection resulting from inoculation followed by immediate planting and by planting after six hours was 21.3 and 19.5 per cent respectively, while only 8.9 per cent occurred when planting was delayed 15 hours. It was evident, therefore, that holding the seed for six hours would not control transmission. The unmottled curly dwarf controls remained healthy but the amount of infection occurring was even less than with spindle tuber, and no definite conclusions can be drawn from the results.

The soil at the time of planting these experiments was quite dry and it is probable that the effect of the dry soil was greater than the effect of holding the tubers for 15 hours after inoculating.

Transmission by picker planters.—The previous experiments which determined that these diseases can be transmitted by the cutting knife and by contact suggested the possibility of transmission by the use of planting machines having small metal devices which puncture the seed pieces in order to pick them up. This possibility was tested in 1928 by the use of small boards with nails driven thru them to simulate the picks of the machine planters. Healthy tubers were quartered, one piece was planted as a control, and the other three pieces were successively punctured by the nail picks which had previously been driven in a similar manner into an infected tuber. The infected seed pieces used as the inoculum, the three seed pieces successively inoculated by

punctures, and the healthy controls were planted in five adjacent rows. Fifty series of inoculations were made with spindle tuber and duplicated with unmottled curly dwarf. Each hill was dug and bagged separately and the tubers were indexed in the greenhouse the following winter. The following percentages of infection are based upon both current-season and perpetuation symptoms.

The seed pieces used as the sources of inoculum produced plants showing typical symptoms of the disease in every instance. All of the healthy controls in the unmottled-curly-dwarf inoculations remained healthy, but two of the 50 controls in the spindle-tuber inoculations became infected. The plants from the inoculated seed pieces showed the following percentages for the first, second, and third punctures respectively: spindle tuber 10, 2, and 0 per cent; unmottled curly dwarf 12, 4, and 0 per cent.

These results indicate that while these diseases may be transmitted in this manner, the amount of infection occurring would be negligible if only a small percentage of the seed tubers were infected. A very small amount of infection occurred in the seed pieces receiving the second puncture and no infection occurred as the result of the third puncture.

Discussion.—In summarizing the various experiments with seed-piece inoculation, it can be stated that both spindle tuber and unmottled curly dwarf can be transmitted by contact of freshly cut seed pieces and by the cutting knife, and that cutting knife transmission can be carried to four successive seed pieces. A treatment of the seed pieces with an organic mercury was effective immediately after inoculation but not six hours after. Drying of the cut surface of seed pieces for six hours before inoculation reduced the amount of transmission. Drying for six hours after inoculation and before planting was not effective in preventing transmission. Holding the inoculated seed for 15 to 24 hours greatly reduced infection and there was some evidence that the moisture conditions of the soil also affected the amount of transmission. With a high soil moisture a high percentage of transmission was obtained, while with a very dry soil a smaller amount of transmission occurred, even when the seed pieces were planted immediately after inoculation. The results of the picker-planter test indicated that such machines transmit spindle tuber to the first and second seed piece but not to the third.

The usual practice of planting potatoes in Nebraska is to carry enough seed over night to start operations the next day. Under usual conditions the cut seed would not have

an opportunity to dry as rapidly as in these experiments, and it is doubtful whether the amount of transmission would be greatly reduced. With only a small amount of disease present in the seed stock, the danger of extensive spread by the usual cutting and planting practices is too slight to warrant special precautions. Where tuber-unit planters are used for either seed plots or commercial fields the danger of transmission is much greater, especially if the soil is in good condition. Certain attachments have been developed for disinfecting the knife and the seed piece at the time of cutting on the planting machine and such a treatment should eliminate the transmission of the diseases.

SPREAD OF VIRUS IN TUBERS AFTER HARVEST

The rate of spread or diffusion of the infectious principle thru the tuber is significant in relation to the incomplete or partial infection of tubers produced by an infected plant, and to the extent of penetration when the freshly wounded or cut surface of a tuber is brought into contact with infectious material. The following experiment, using seed-piece plug inoculations, was conducted to obtain information on this point.

Five series of inoculations of ten tubers each were made in healthy Triumph tubers weighing 250 to 300 grams. Two of the ten tubers of each series were planted 2, 4, 8, 16, and 18 weeks after inoculation. At the time of inoculation one seed piece was removed from the tuber as a control. In making the inoculations a core of tissue 12 mm. in diameter, obtained from an infected tuber was forced into a slightly smaller cavity in the healthy tuber. In some series the inoculations were made at the basal-end and in others at the apical-end of the tuber. At the time of planting the tuber from which one seed piece had already been removed was cut into 5 seed pieces by one cut lengthwise and two across. The position of each seed piece before cutting was recorded and each plant produced was harvested separately.

Sixty-one of the 70 seed pieces containing the inoculation plug produced infected plants. The percentage of infection from the other seed pieces of each tuber was very low, even from those adjoining the portion of the tuber inoculated. The cut separating the inoculated portion from the adjoining one was often only 5 to 10 mm. from the inoculation plug and parallel to it. Even with this close proximity to the inoculation plug only 6 out of 71 seed pieces produced infected plants. From those portions further removed from the inoculation only six infections occurred from 94 seed pieces.

There was no significant difference in the results from the apical-end as compared with basal-end inoculations. Likewise, there was little difference resulting from the different intervals of time between inoculation and planting. In fact, the only set that did not produce any infected plants was that in which the inoculation core remained in the tuber the greatest time, 18 weeks.

In this test the inoculated tubers were held in cold storage at 2.5° to 5° C. until planted, and a rapid spread of the virus thru the tuber would not be expected under these conditions where the life processes of the tuber were at a minimum. The tubers in the last two plantings were removed from storage and shipped 400 miles to the field in late May, so that all the sprouts were well developed at the time of planting. From these tests it is conceivable that tubers which occasionally produce both healthy and infected plants may have become infected late in the preceding season, and that the virus had not spread thru the entire tuber before harvest and did not continue to spread after harvest. It would also appear that the contact of freshly cut surfaces of healthy and infected tubers at harvest or in storage would not result in any great spread of the disease.

THE EFFECT OF VARIOUS TREATMENTS UPON THE VIRUS OF SPINDLE TUBER AND OF UNMOTTLED CURLY DWARF

A study of the effect of various chemical and physical treatments of the virus is of importance in obtaining a better understanding of the nature of the virus, and the information obtained often serves as a basis for methods of distinguishing one virus from another, or for the separation of mixed viruses. The results also have a possible application in the development of control methods and are important in determining the methods to be employed in other phases of the virus problem. Such studies have been carried out with a large number of virus diseases, but very little information is available concerning those of the potato and none regarding spindle tuber and unmottled curly dwarf.

Studies of this phase of the problem were started some years ago but were discontinued before definite and conclusive results could be obtained. The following results obtained from these tests may be somewhat changed, especially as regards those of a negative nature, when larger and more comprehensive tests are conducted.

Dilution.—Experiments were conducted to determine the effect of dilution upon the infectivity of the extracted juices of spindle-tuber and unmottled-curly-dwarf plants. Three

series of tests were made, in the greenhouse in 1925 and in the field in 1927 and 1928. Essentially similar methods were used in each test. The inoculum was obtained by crushing the foliage of infected plants in a mortar and filtering the juice thru cheesecloth, after which it was diluted to the desired degree with tap water. Healthy plants were grown in tuber-units and cut back to a single stem, one plant of each unit being kept as a control and the others inoculated by inserting a small piece of cotton saturated in the juice into small wounds made in the axils of the three youngest petioles. A record was made of the development of current-season symptoms and of the perpetuation symptoms the following year. The results are summarized in Table 8. Some reduction in the percentage of infection occurred with all dilutions. There was a sharp drop in the percentage of infection with spindle tuber when the juice was diluted 1 : 10,000; in fact, the one infection obtained is questionable, as the controls showed 8.5 per cent infection due to uncontrolled current-season transmission. The negative results with a 1 : 100

TABLE 8.—*The effect of dilution upon the infectivity of the juice extracted from the foliage of spindle-tuber and unmottled-curly-dwarf plants*

Dilution with water	Spindle tuber			Unmottled curly dwarf		
	Inoculated plants	Infected plants		Inoculated plants	Infected plants	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
Undiluted	15	15	100.0	7	7	100.0
1—10	9	7	77.7	..	..	.....
1—100	2	0	0.0	10	6	60.0
1—1,000	22	11	50.0	29	3	10.3
1—10,000	22	1	4.5	30	2	6.6
1—100,000	20	0	0.0	30	1	3.3
Controls	35	3	8.5	44	1	2.2

dilution were probably due to the small number of tests made, as 50 per cent infection was obtained with a dilution of 1 : 1,000. The virus of unmottled curly dwarf did not appear to be as tolerant to dilution, as there was a drop from 60 per cent with a 1 : 100 dilution to 10 per cent with a 1 : 1,000 dilution. Some infection occurred with the higher dilutions but the small number of instances combined with a slight amount of infection in the controls make the results questionable. The evidence from these few tests is not sufficiently clear to serve as a basis for separating these two viruses. These two viruses, particularly spindle tuber, appear to tolerate a slightly greater dilution than the four potato mosaic viruses reported upon by Johnson (10). It is questionable, however, whether the dilution

method would serve as an accurate means of separating the viruses, altho additional tests with more accurate and standardized methods might make this possible.

Effect of heat upon the virus.—In 1927, R. W. Samson, working in this laboratory, reported[1] on the effect of heat upon the infectiousness of spindle-tuber tissues. The technique he employed was somewhat different from that usually used in determining thermal death points, in that he used tuber tissue in place of extracted juice. In his tests cores of tissue 12 mm. in diameter and about 4 cm. long were removed from spindle-tuber potatoes and placed in uniform test tubes containing 10 c.c. of water. They were held in a water bath at the desired temperature for 10 minutes and then used to inoculate healthy tubers by inserting the core of tissue into a hole in the healthy seed piece. The temperatures were recorded by inserting a thermometer into the center of a control core of tissue submitted to the same experimental conditions. The time required for the core to reach the desired temperature and the time required for the temperature to drop to 40° C. after removal from the water bath were also recorded. He found the following percentages of infection to occur, using 5 to 10 inoculations for the tests at each temperature: 83 per cent at 40°, 83 per cent at 45°, 50 per cent at 50°, 25 per cent at 55°, 18 per cent at 60°, and no infection at 65° or 70° C. At 65°, the lowest temperature at which infection did not occur, Samson reports that it required 4.25 minutes for the center of the core of tissue to reach that of the water bath. It was held at this temperature 5.75 minutes, or a total of 10 minutes in the bath, and it required 9 minutes for the tissue to cool down to 40°. The author further checked Samson's results by indexing the progeny of his inoculated plants the following year. His results indicate that the thermal death point for spindle-tuber potato tissue, when treated by this procedure, lies between 60° and 65° C.

In 1928 the effect of heat upon the virus of unmottled curly dwarf was determined by the usual procedure of extracting the plant juice and submitting a definite amount, 7.5 c.c., to a series of temperatures in a water bath for 10-minute periods. Test tubes with walls of uniform thickness were used and the water bath was manipulated so that in each test four minutes was required to bring the extract in the tubes to the desired temperature. After a 10-minute exposure they were

[1] Samson, R. W. A Study of the Properties and Nature of the Virus of the Spindle Tuber Disease of Potatoes. Unpublished thesis. June, 1927. University of Nebraska Library.

then cooled, so that it took just 15 minutes for the temperature to reach 30° C. The juice was then inoculated into young, healthy plants by the insertion of cotton saturated in the extract into three incisions in the main stem at the axils of the three youngest petioles. Ten to fifteen inoculations were made for the tests at each temperature. Thirty per cent infection was obtained after exposure to a temperature of 75° but no infection resulted from the 85° test. The thermal death point, as here tested, evidently lies above 75°, which is 10° to 15° above the thermal death point determined for spindle-tuber tissue by Samson.[1] It is impossible, with the different technique employed in these separate tests of the two diseases to accurately compare the results, particularly as regards the length of exposure at the critical temperature. It is evident, however, that the thermal death points for the viruses of spindle tuber and unmottled curly dwarf are higher than for crinkle mosaic and mild mosaic and approximately the same as for rugose mosaic and leaf-rolling mosaic as reported by Johnson (10).

Effect of aging in vitro and desiccation in plant tissue.—The effect of aging was tested by obtaining the juice from freshly cut foliage of infected plants, and after storing under toluol at 10° C. for definite periods the juice was used to inoculate healthy plants. No infection resulted from any of the inoculations, the first of which, six inoculations with each disease, were made 24 hours after extraction. The experiment was not repeated, so that no definite results can be drawn, owing to the lack of infection, other than that the virus of both diseases is apparently inactivated rapidly after being extracted from infected plants.

The effect of desiccation was tested by cutting and drying the foliage of spindle-tuber plants at room temperature and then grinding with a small amount of water and inoculating into healthy plants by the leaf mutilation method in the greenhouse. Infection was obtained after one week's drying but not after 17 days or longer periods. The number of plants used was too small to determine the limits definitely, but it was evident that the virus was much more resistant in the dried host tissue than *in vitro*.

Attempted cultivation of spindle-tuber virus.—An effort was made in 1926 to determine whether the virus could multiply in sterile tissue removed from the host plant by using a modification of the methods employed by Olitsky (16), who reported positive results with tobacco mosaic, and Mulvania (13) and Goldsworthy (3), who obtained negative results with the same disease.

[1] *Op. cit.*

Cores of healthy tubers were removed aseptically and immersed in 10 c.c. of sterile water in test tubes. Similar cores of spindle-tuber potatoes were used in other tubes. After incubation for two days at 25° C., one c.c. of the liquid in a tube of spindle-tuber tissue was transferred to a tube of healthy tissue. Transfers were subsequently made every 24 hours to new tubes of healthy tissue. After each transfer the core of tissue from the tube inoculated the previous day was used to plug into the cavities of healthy seed pieces. The cultures made in this way resulted in a series of dilutions ranging from 1:10 up to $1:10^{11}$. The test was run in triplicate.

None of the plants grown from the inoculated seed pieces developed the disease.

Tests on soil transmission.—No evidence has been found, in the few tests conducted in the greenhouse, of the ability of the virus to exist in the soil. Healthy seed planted in soil previously containing spindle tuber and unmottled curly dwarf plants have always produced healthy plants.

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